

## **EXHIBIT D**

From Page No. 34

Prepare hybridization mixes

lysates:

30ul DIBSS diluent

10ul lysate

360ul DIBSS / probe

400ul

Do duplicate

Pos. Control

30ul DIBSS

1ul Chlamydia RNA ~ 10ul E. coli RNA

+ 9ul H<sub>2</sub>O

360ul DIBSS / probe

400ul

Incubate 1hr 64°C

Neg. control

30ul DIBSS

10ul 3.2% SDS

360ul DIBSS / probe

400ul

Add 4.5ml Separation Solution

.14M PB LN 60278

Incubate 5' at 64°C

Centrifuge

Decant SN into 30ml scintillation vial

Add 5.0ml Wash solution

LN 60276 .14M PB

Incubate 5' at 64°C

Centrifuge

Decant SN into same scintillation vial as 1st wash

1. Count <sup>HA</sup> pellets and wash fraction for <sup>32</sup>P counts

2. Add 5.0ml cytochrome to HA pellet

Add 15.0ml gel to wash vial

Count again in gel <sup>125</sup>I counts

Used Sal for counting 2x2'

Prepare blanks ① spin down 4.5 ml HA and count

② add 9.0ml .14M wash and to scint. vial and count

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Witnessed & Understood by me,

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TITLE

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From Page No. 38

Purpose: To assay lysates of Chlamydia strains for cross reactivity with probes at 60°C and 176 at 64°C.

Reagents: Same as for previous experiment (405:34)

In addition used lysates for C. trachomatis 300, 301, 302, 303 (other serotypes than the one used to make the probes and found in humans)

From Mary NB 435:3

Diluted 711 RNA 25 µg/µl (334:10) 1.5 to 105 µg/µl

Made up probe solutions

- ① To 8.4 µl DIBS3 mix, add 5 µl of Probe 176 ( $1.3 \times 10^6$  cpm/µl) NB 391:25 kinase and 20 µl of Probe 1082 ( $22,000$  cpm/µl) iodinated by Kevin NB 388:42

②

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From Page No.     

Assay Protocol: Prepare hybridization mixtures in scintillation vials:

Lysates: 30  $\mu$ l DIBSS diluent  
 10  $\mu$ l lysate  
 360  $\mu$ l DIBSS/probe mix  
 400  $\mu$ l total volume

N.C.: 30  $\mu$ l DIBSS diluent  
 10  $\mu$ l 3.2% SDS  
 360  $\mu$ l DIBSS/probe mix  
 400

711 RNA: 30  $\mu$ l DIBSS diluent  
 2  $\mu$ l RNA 105  $\mu$ g/ml  
 8  $\mu$ l H<sub>2</sub>O  
 360  $\mu$ l DIBSS/probe  
 400

E. coli RNA: 30  $\mu$ l DIBSS diluent  
 10  $\mu$ l RNA 105  $\mu$ g/ml  
 360  $\mu$ l DIBSS/probe  
 400

Incubate ChtA 176 probe rxn at 64°C 1 hr.

Separate hybrids and wash AB 405 as p. 35

Count in HAL 2x2' on  
 to get <sup>32</sup>P counts Program 1

Add 0.09 ml of probe mixture to HA pellet in scint vial

Add 5.0 ml cytoscent and count use this value  
 for total counts for <sup>125</sup>I-hybridization w/ pan bacterial probe

Add 5.0 ml cytoscent, and count in HAL 2x2'  
 to get <sup>125</sup>I counts. ~~etc~~ Program 1 p. 405:34

(Did not count washes for <sup>125</sup>I as did in previous expt)

E. coli RNA sample 2 was spilled for probe ChtA 176.

Results: see p. 41

Conclusions: Probes 176 and reached with all C. trachomatis  
 strains but not with the C. psittaci. The <sup>125</sup>I-E7082  
 data showed that RNA was present in all tubes

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TITLE Specificity Study of Chlamydia probes

Project N 3  
B k N 405

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		CAT A 176 EcoA N12	32p		
	N	SMPL	CPM	TOTAL	%
711	1	FP	929.8	2391.8	44.5
		BP	1161.3		55.5
	2	FP	942.3	2166.8	43.5
		BP	1223.8		56.5
300	3	FP	623.0	2210.0	28.2
		BP	1587.0		71.8
	4	FP	631.8	2179.3	29.0
		BP	1547.5		71.9
301	5	FP	561.5	2180.3	25.8
		BP	1618.8		74.2
	6	FP	536.8	2159.5	24.9
		BP	1622.8		75.1
302	7	FP	566.5	2147.5	26.4
		BP	1581.0		73.6
	8	FP	610.8	2248.3	27.2
		BP	1637.5		72.8
303	9	FP	551.3	2178.0	25.3
		BP	1626.8		74.7
	10	FP	563.5	2124.0	26.5
		BP	1560.5		73.5
794	23	FP	2278.5	2295.0	99.7
		BP	16.5		.7
	12	FP	2313.5	2328.0	99.4
		BP	14.5		.6
767	13	FP	2228.5	2239.3	99.5
		BP	10.8		.5
	14	FP	2284.0	2295.5	99.5
		BP	11.5		.5
768	15	FP	2303.3	2319.5	99.3
		BP	16.3		.7
	16	FP	2302.5	2318.3	99.3
		BP	15.8		.7
711 RNA	17	FP	403.0	2152.0	18.7
		BP	1749.0		81.3
	18	FP	310.0	2073.8	14.9
		BP	1763.8		85.1
coli	19	FP	2202.8	2217.3	99.3
		BP	14.5		.7
	20	FP	590.8	597.3	98.9
		BP	6.5		1.1
pc	21	FP	2298.5	2316.3	98.9
		BP	25.8		1.1
	22	FP	2255.3	2278.0	99.0
		BP	22.8		1.0

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Results:

ECOR 1082 with 176

TOTAL 1: 13760

SAMPLE	CPM	HAZ	AVG%	XBKGD
711	5849	49.8		
	7170	52.1	50.9	
300	8420	61.2		
	8571	62.3	61.7	
301	9490	69.0		
	9610	69.8	69.4	
302	8809	64.0		
	9557	69.5	66.7	
303	9153	66.5		
	8793	63.9	65.2	
754	7520	54.7		
	7666	55.7	55.2	
767	7692	55.9		
	7477	54.3	55.1	
768	4677	34.0		
	4483	32.6	33.3	
Av. 711	8877	64.5		
	9516	69.2	66.8	
Coli.	8431	61.3	61.3	
N.C.	139	1.0	1.0	
	132	1.0	1.0	

Lysates		<sup>32</sup> P-CAT A 176	% Hybridization <sup>125</sup> I-Eco A 1082
C. trachomatis	711	56.0	50.9
"	300	71.4	61.2
"	301	74.5	69.4
"	302	73.2	66.7
"	303	74.1	65.2
C. psittaci	754	0.7	55.2
"	767	0.5	55.1
"	768	0.7	33.3
RNA			
C. trachomatis	711	83.2	66.8
E. coli		0.9	61.3
N.C.		1.1	1.0

Hybridization  
Assays done at 60°C

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